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(54) Title: LIPOSOMAL FORMULATIONS CONTAINING RIFAMYCINS (57) Abstract <p>The invention relates to liposomal formulations containing a rifamycin and/or a hydrophobic derivative as a pharmacological active substance, for clinical use, comprising pharmacological active substance/lipid ratios of 1 mole per 10 to 40 moles, lipidic compositions made by mixtures of glycerophospholipids of saturated or unsaturated acyl chains, with cholesterol and charged molecules of lipidic nature or not. The liposomal rifamycin presents a stability in saline and in human serum higher than 50 %, incorporation efficiencies up to 95 %, intraliposomal concentration of at least 1 mg/ml and toxicity lower compared to the free pharmacological active substance. The invention also refers to a process for the preparation of liposomal formulations comprising solving the pharmacological active substance and the other components in appropriate solvents, lyophilization or not and rehydration of the lyophilized or resulting mixture.</p>		

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DESCRIPTION

LIPOSOMAL FORMULATIONS CONTAINING RIFAMYCINS

This invention relates to the preparation of liposomal formulations containing a rifamicin and mixtures of glycerophospholipids of saturated or insaturated acyl chains, with cholesterol (Chol) and charged molecules, lipidic or not (e.g. stearylamine (SA), phosphatidic acid (PA)).

The rifamicins are a group of antibiotics where rifampicin is included. It is one of the most active tuberculostatic used in clinic. Its activity may be increased by liposomal encapsulation because the antibiotic pharmacokinetic becomes changed. When liposomes are administered I.V. (intravenously) they are taken up by macrophages (1 - 3). So, these therapeutic systems may be used for carrying drugs to the infected macrophages increasing, by this way, the antibiotic concentration where it is needed and reducing it where the drug might be toxic. Concomitantly some of the antibiotics of this group evidence an anti-virus action which may be increased by administration in liposomal form.

It is possible to modulate the in vivo behaviour of liposomes by changing the lipidic composition, size or charge of these vesicles.

Macrophages make part of the reticuloendothelial system being very important cells for the organism defense. The macrophage functions are phagocytosis and destruction of organism foreign bodies. However, there are some organisms able to survive inside these cells and being very difficult to eliminate, namely Mycobacterium Tuberculosis, Mycobacterium Avium and other organisms sensible to the rifamicins (4). The

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present invention is addressed to the problem of preparing liposomes with efficient incorporation of Rifampicin. Rifampicin has been incorporated in liposomes before (5 and 6). To obtain a suitable and better liposomal formulations the following characteristics are required: preservation of Rifampicin activity, high incorporation efficiency, high stability and reduced toxicity.

It has been found that liposomal formulations with the above referred requirements are those which have the parameters and prepared by the methods described hereinafter.

The present invention relates to a process of liposomal formation by lyophilization followed by hydration of the lyophilized powder. This process comprises the following steps:

- Formation of a solution containing the antibiotic and the liposomal lyophilic components, in appropriate solvent or solvent mixtures like t-Butanol or cyclohexane. Appropriate solvent or solvent mixtures means that Rifampicin and other components should form an homogeneous solution which can be lyophilized.

- Solidification of solution by freezing.

- Lyophilization of the solid form.

- Hydration of lyophilized powder with water, saline or any other appropriate aqueous solution.

In prior methods described in the literature although using rifampicin and lipid concentration in the same range of the present invention the maximal I.E. (Incorporation Efficiency) obtained was 76% (5). In the present invention this parameter goes up to 95%.

The methods used by several authors (5 - 9) are different from that of the present invention, although similar methods have already been used for other drugs (10). In methods described in the literature no lyophilization steps are used (5, 6, 8 and 9). Lyophilization of lipid and drug mixture, followed by hydration, as done in the present process, allows a higher drug incorporation into the lipidic matrix of liposomes.

Also the lipids described in the literature are different

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from those of the present invention, where lipids with phase transition temperature (T_c) higher than 37°C were found to be more appropriate: dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), and distearoylphosphatidylcholine (DSPC). There are no data in the literature concerning stability in saline and human serum of Rifampicin liposomal formulations.

Some stability tests in human serum and liposomal incorporation were made by using rifampicin, cyclophen-tylrifampicin and rifabutin.

It is possible to incorporate rifamicins in liposomes with different lipidic compositions. However, the lipidic compositions which showed higher stability in human serum, are those whose main lipid component is a phospholipid with a T_c higher than 37°C . The stability in human serum is crucial, so liposomes can keep their characteristics after administration. Liposomes with low stability in human serum release rifamicins at disruption step, behaving the antibiotic as free drug and so ceasing the advantages of preparing a liposomal form.

Toxicity tests were made comparatively with free and liposomal rifampicin by using several models: chinese hamster ovarium cells (CHO) as a model for non phagocytic cells toxicity; superoxide anion release from macrophages as a model for phagocytic cells toxicity and potassium release from erythrocytes as a model for membrane toxicity. The acute toxicity was tested in mice.

The liposomal rifampicin showed to be less toxic than the free rifampicin for the chinese hamster ovarium cells. This formulation did not show to suppress O_2^- production by macrophages, showing an induction effect on superoxide ion production. This is very important because the production of this ion allies to the hydrogen peroxide, a compound used by the human body to fight against infection caused by bacteria. The liposomal rifampicin did not show any induction effect on the potassium release by the erythrocytes. Also no destabilizing activity on the membranes was evidenced. Acute toxicity, made on mice, did not show higher toxicity in liposomal rifampicin compared to free rifampicin.

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The results of the present invention are much improved in terms of Incorporation Efficiency, stability and reduction of toxicity, being appropriate for future clinical use.

CHEMICAL AND BIOLOGICAL CHARACTERIZATION:

Here are presented chemical and biological characterization of this invention for liposomal Rifampicin made from different lipid compositions.

The Incorporation Efficiency (I.E.) means the percentage of liposomal to initial Rifampicin concentration, after previous disruption of liposomes with ethanol.

Encapsulation of rifampicin in liposomes

Table I shows the effect of lipid composition on incorporation of Rifampicin in multilamellar vesicles. The higher I.E. were obtained with the lipids with lower phase transition temperature (T_c): phosphatidylcholine (PC), dioleoylphosphatidylcholine (DOPC) and diphitanoylphosphatidylcholine (DPhPC). The lower I.E. were obtained with lipids having higher T_c : dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC). The inclusion of SA at a molar ratio of 1 to 9 of phospholipid improved the I.E. when compared with the correspondent neutral liposomes (prep. 1, 4 and 5).

To study the effect of anionic lipids on incorporation, a series of experiments was carried out using different phosphatidylglycerols combined at different molar ratios with phosphatidylcholines (prep. 10-20). The addition of phosphatidylglycerols at low molar ratio (1 phosphatidyl-glycerol to 9 phosphatidylcholine) did not improve significantly the I.E. of liposomes made from phosphatidyl-cholines. However, the use of higher molar ratios of phosphatidylglycerols, 3:7 and 1:1, led to preparations with significantly higher I.E. (prep. 11 and 12 vs 1, 15 and 16 vs 4, 19 and 20 vs 5). The use of pure phosphatidylglycerol led to I.E. significantly higher than those obtained with the correspondent phosphatidylcholines. The

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inclusion of cholesterol did not affect significantly the I.E. of formulations tested except for PC: SA (prep. 25 vs 7).

A direct relationship between bilayer fluidity and I.E. was observed in the neutral, cationic and anionic formulations studied (Fig.1). This effect was observed in the series of lipid compositions where liposomes were made using lipids with the same polar heads but different acyl chains.

Stability of liposomal rifampicin in saline

The stability in saline of several formulations was studied at 4°C and at 37°C. The first test was designed to evaluate the behaviour of the vesicles during storage and the second to study the stability of the liposomes at a physiological temperature. The compositions containing phosphatidylglycerols alone or mixed with phosphatidylcholines were the most stable at 4°C after 15 days, with 92 to 98% of rifampicin retention. Inclusion of Chol did not improve drug retention. The compositions containing SA were the least stables, with 60 to 75% retention. The decrease in stability in all formulations was more pronounced in the first 24 hours, (burst like effect) than in the following 15 days.

At 37°C rifampicin retention values between 82 to 100 %, after 48 hours of incubation, were observed. Dimiristoyl-phosphatidylcholine (DMPC): dimyristoylphosphatidylglycerol (DMPG) (1:1) and phosphatidylglycerol (PG) showed the highest stability (respectively 95 and 100%).

Stability in serum at 37°C

The formulations that presented high stability in saline and high I.E. had a low stability in serum (Table II). Chol did not improve the stability of the formulations where this lipid was included. Higher stability was observed for vesicles made from lipids with a Tc higher than 37°C.

Effect of lipid concentration

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In order to optimize the incorporation of rifampicin in liposomes with high serum stability, we studied the effect of lipid concentration on rifampicin incorporation in vesicles made with DPPC or DSPC. I.E. can be improved significantly if lipid concentration increases to 60 $\mu\text{mol/ml}$ (Fig.2).

Incorporation of rifampicin versus drug/lipid ratio

The variation of I.E. and total rifampicin incorporation as function of the drug/lipid (D/L) ratio used in DPPC liposomes was also studied (Fig. 3). Although total rifampicin incorporation increases with the increase in D/L ratio, the I.E. decreases with it. 1.5 μmol rifampicin/60 μmol lipid was chosen as the ideal D/L ratio. Vesicles with I.E. of $86 \pm 3\%$ and incorporation of drug of 1.2 μmol (1 mg) of rifampicin per ml of liposome suspension can be obtained at this ratio. In experiments where vesicles highly loaded with antibiotic are needed (e.g. mice toxicity) higher D/L ratios can be used.

Characterization of serum stable liposomes

Two formulations including DPPC were compared: DPPC and DPPC:DPPG (9:1). Both formulations were made using 60 μmol lipid/ml and D/L ratio of 1.5 μmol rifampicin/60 μmol lipid. They showed similar saline and human AB serum stability at 37°C (Fig.4).

The I.E. of rifampicin is also comparable ($86 \pm 3\%$ for DPPC vs $84 \pm 1\%$ for DPPC:DPPG (9:1)). In relation to size distribution and microscopic structure the vesicles made from DPPC:DPPG showed a narrower size distribution (90% between 2-5 μm) and no clumps of liposomes were observed.

This last formulation was used for all in vitro and in vivo experiments.

Toxicity to CHO cells

At therapeutic concentrations (10 μM) there was no

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difference between free and liposomal rifampicin with respect to cells (chinese hamster ovarium) toxicity. To see significant differences, concentrations higher than 200 μM were required. At concentrations of free rifampicin higher than 450 μM , greater than 95% cytotoxicity was observed, however liposomal rifampicin was less toxic (Fig. 5).

Toxicity to macrophages

By gross morphology and trypan blue exclusion, discernible toxicity to macrophages was not observed. After overnight exposure macrophage monolayers showed no apparent morphological changes. Treated cells were well spread, looked normal and did not take up trypan blue to a greater extent than controls. During the observation with liposomal rifampicin or empty liposomes some liposomes could be seen inside the macrophages.

We next assessed the effect of free or liposomal rifampicin on superoxide anion (O_2^-) release by macrophages (Fig. 6). Cells exposed to free rifampicin had a decrease in production of O_2^- at doses higher than 5 μM . However when encapsulated in liposomes, rifampicin at concentrations up to 80 μM did not show any inhibition on the O_2^- production when compared to a control medium and had a little induction effect. Empty liposomes are not statistically different from liposomal rifampicin values.

Red Blood Cells toxicity

We also assessed the effect of free and liposomal rifampicin on K^+ release by red blood cells (RBC). K^+ content of incubation medium of RBC with free and liposomal rifampicin was not significantly different from incubation medium of RBC with free drug or from the control incubated with saline and RBC (80 μM K^+). Control incubated with water had a value of 1154 μM K^+ .

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In vivo toxicity

For animal experiments doses of 10, 85, 170 and 340 mg/kg of free and liposomal rifampicin were injected to groups of 4 mice each. Liposomes with rifampicin were made using drug/lipid ratio of 2:10 and lipid concentration of 60 μ mol/ml of lipid. At the dose of 10 mg/kg (usual therapeutic dose) we did not notice any alterations on behaviour of the mice. At higher doses (9-34 fold the therapeutic dose) the mice became hiperactive, but after a while behaved normally until the end of the experiment. At the highest dose the skin of the mice acquired an orange colour due to the high rifampicin concentrations either in free or liposomal rifampicin groups. One mice of the dose 170 mg/kg of free rifampicin and other of the dose 340 mg/kg of liposomal rifampicin died. Higher doses were not tested due to practical difficulties of injecting volumes greater than 1 ml of a concentrated suspension of lipid particles and of solubilizing rifampicin in the vehicle used. In control groups (vehicle and empty liposomes) there were no dead animals.

TABLE I Effect of lipid composition on Incorporation Efficiency of rifampicin in liposomes

PREPARATION NUMBER	LIPID COMPOSITION (molar ratio)	I.E. (%)
Effect of fluidity		
1	PC	66 \pm 5
2	DOPC	67 \pm 2
3	DPhPC	68 \pm 2
4	DMPC	57 \pm 6
5	DPPC	50 \pm 7
6	DSPC	45 \pm 4

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Effect of stearylamine

7	PC:SA (9:1)	84±3
8	DMPC:SA (9:1)	74±4
9	DPPC:SA (9:1)	68±3

Effect of phosphatidylglycerol

10	PC:PG (9:1)	75±9
11	PC:PG (7:3)	91±6
12	PC:PG (1:1)	97±2
13	PG	98±2
14	DMPC:DMPG (9:1)	62±5
15	DMPC:DMPG (7:3)	74±4
16	DMPC:DMPG (1:1)	96±3
17	DMPG	86±11
18	DPPC:DPPG (9:1)	50± 5
19	DPPC:DPPG (7:3)	71± 3
20	DPPC:DPPG (1:1)	87± 2

Effect of cholesterol

21	PC:PG:Chol (7:3:5)	80± 5
22	DMPC:DMPG:Chol (7:3:5)	65± 7
23	PC:PG:Chol (1:1:0.6)	95± 3
24	PG:Chol (10:3)	96± 4
25	PC:SA:Chol (9:1:2)	73± 4

Liposomes were prepared with 16 μ mol lipid/ml and 1:10 ratio of rifampicin:phospholipid.

The number of experiments ranged between 3 and 6. Mean values and standard deviations are given. The data were analysed by the Student's t test, with a confidence interval for difference in means of 95% ($\alpha = 0,05$). Pure phospholipids have the following Tc values ($^{\circ}$ C): PC (-15 /-17); PG (-15/17) DOPC (-22); DMPC (23); DPPC(41); DSPC(55); DMPG(23); DPPG(41).

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TABLE II. Stability of liposomes in human AB serum at 37°C

Stability	Lipid composition	
< 25 %	DMPC:DMPG 1:1	PG
	PC:PG 1:1	PG:Chol 10:3
	PC:PG:Chol 1:1:0.6	PG:Chol 1:1
25-50 %	DPhPC	DPPC:DPPG 1:1
	DEPC	
	DOPC	
50-75 %	DPPC:DPPG 7:3	
>75 %	DPPC	DPPC:SA 9:1
	DSPC	
	DPPC:DPPG 9:1	

Liposomes were prepared with 16 μmol lipid/ml and 1:10 ratio of rifampicin:phospholipid.

DESCRIPTION OF FIGURES

FIG.1 shows the effect of the liposome fluidity in rifampicin incorporation in liposomes.

FIG.2 shows the effect of lipid concentration on rifampicin incorporation in liposomes made from DPPC (Δ) or DSPC (*). MLV were made using 1.6 $\mu\text{mol}/\text{ml}$ of rifampicin and lipid concentrations ranging from 16 to 70 $\mu\text{mol}/\text{ml}$ of lipid.

FIG.3 shows the variation of I.E. (*) and total rifampicin incorporation (Δ) as function of the drug/lipid (D/L) ratio used in DPPC liposomes. Liposomes were made using 60 $\mu\text{mol}/\text{ml}$ of lipid and rifampicin concentrations ranging from 6 to 7.5 $\mu\text{mol}/\text{ml}$.

FIG.4 shows the stability in saline (full line) of liposome compositions DPPC (*) and DPPC:DPPG 9:1 (\square) and stability in human serum (dotted line) of liposome compositions

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DPPC (Δ) and DPPC:DPPG 9:1 (□). The essays were done at 37°C during 48 hours.

FIG.5 shows the cytotoxicity of free (*) versus liposomal (Δ) rifampicin to CHO cells as determined by MTT test. Empty liposomes were used as control, with cytotoxicity values of $100 \pm 3\%$.

FIG.6 shows the release of O_2^- by mouse peritoneal macrophages after incubation with free rifampicin (□), liposomal rifampicin (Δ) or empty liposomes (*) at the lipid concentrations used for liposomal rifampicin.

FIG.7 shows the chemical structure of Rifampicin.

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CLAIMS

1. Liposomal formulations or their mixtures that after hydration are able to form liposomes, containing at least one rifamicin and/or an hydrophobic derivative as pharmacological active substances, characterized by having stability higher than 50%, incorporation efficiency up to 95% and an intraliposomal concentration of at least 1 mg per ml of liposome suspension.
2. Liposomal formulations according to claim 1, characterized by having a stability in saline at 4°C higher than 50% after 15 days of incubation.
3. Liposomal formulations according to claims 1 and 2, characterized by having a stability in human serum at 37°C higher than 50% after 48 hours of incubation.
4. Liposomal formulations according to claims 1 to 3, characterized by the fact that liposome diameters range from 0.05 μm to 10 μm .
5. Liposomal formulations according to claims 1 to 4, characterized by the fact that liposomes have on their composition synthetic and/or natural phospholipids.
6. Liposomal formulations according to claims 1 to 5, characterized by using mixtures containing phospholipids of saturated and/or insaturated acyl chain.
7. Liposomal formulations according to claims 1 to 6, characterized by the fact that they contain phospholipids with phase transition temperature higher than 24°C.
8. Liposomal formulations according to claims 1 to 7, characterized by the fact that they also contain neutral or

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charged molecules of non lipidic nature.

9. Liposomal formulations according to claims 1 to 8, characterized by the fact that they also contain cholesterol.

10. Liposomal formulations according to claims 1 to 9, characterized by the fact that they contain at least one of the following phospholipids: phosphatidylcholine (PC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), diphitanoylphosphatidylcholine (DPhPC), phosphatidylglycerol (PG), dimyristoylphosphatidylglycerol (DMPG), dipalmitoylphosphatidylglycerol (DPPG) and distearoylphosphatidylglycerol (DSPG).

11. Liposomal formulations, according to claims 1 to 10, characterized by the use of one or more of the following pharmacological active substances: rifampicin, rifabutin, cyclopentylrifampicin or any other rifamicin or even their hydrophobic derivatives.

12. Process for preparing liposomal formulations, according to claim 1, characterized by the following steps:

- Lipid and pharmacological active substance solubilization in a solvent that remains in the solid state during the lyophilization process.
- Freezing the resulting solution.
- Lyophilization of the resulting solid form.
- Hydration of the lyophilized powder with water or any other appropriate aqueous solution, immediately after their lyophilization or before administration.

13. Process for preparing liposomal formulations according to claim 1, characterized by the fact that liposomes are obtained by hydration of non lyophilized form of phospholipids.

14. Process according to claim 12, characterized by the fact that the pharmacological active substance is added to the

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lyophilized powder.

15. Process according to claims 12 to 13, characterized by the fact that the pharmacological active substance is added in hydration step.

16. Process according to claims 12 to 15, characterized by the fact that the hydration aqueous solution contains other pharmacologically active substance.

17. Process according to claims 12 to 16, characterized by using synthetic and/or natural phospholipids.

18. Process according to claims 12 to 17, characterized by using mixtures containing phospholipids of saturated or insaturated acyl chains.

19. Process according to claims 12 to 18, characterized by the fact that it contains phospholipids with phase transition temperature higher than 24°C.

20. Process according to claims 12 to 19, characterized by also using cholesterol.

21. Process according to claims 12 to 20, characterized by using also neutral or charged molecules of non lipidic nature.

22. Process according to claims 12 to 21, characterized by the fact that it contains at least one of the following phospholipids: phosphatidylcholine (PC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), diphitanoylphosphatidylcholine (DPHPC), phosphatidylglycerol (PG), dimyristoylphosphatidylglycerol (DMPG), dipalmitoylphosphatidylglycerol (DPPG) and distearoylphosphatidylglycerol (DSPG).

23. Process according to claims 12 to 22, characterized by

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using a pharmacological active substance/lipid molar ratio ranging from 1/3 to 1/80, preferably 1/10.

24. Process according to claims 12 to 23, characterized by using a lipidic film concentration, to form the liposomal formulation, of at least 4 mM.

25. Process according to claims 12 to 24, characterized by using, to form the liposomal formulations, a pharmacological active substance in a concentration of at least 1 mg/ml of solution.

26. Process according to claims 12 to 25, characterized by having an encapsulation efficiency up to 95%.

27. Process according to claims 12 to 26, characterized by the fact that the pharmacological active substance here used might be rifampicin, rifabutin, cyclopentylrifampicin or any other rifamicin or their hydrophobic derivatives.

28. Process according to claims 12 to 27, characterized by the fact that the so obtained liposomes are sized by extrusion.

29. Process according to claims 12 to 28, characterized by the fact that the non-incorporated pharmacological active substance is separated from liposomes.

30. Process according to claims 12 to 29, characterized by obtaining liposomal formulations with reduced in vitro toxicity compared to free pharmacological active substance and no acute toxicity.

31. Liposomal formulations according to any of claims 1 to 11, characterized by being prepared by the process according to any of claims 12 to 30.

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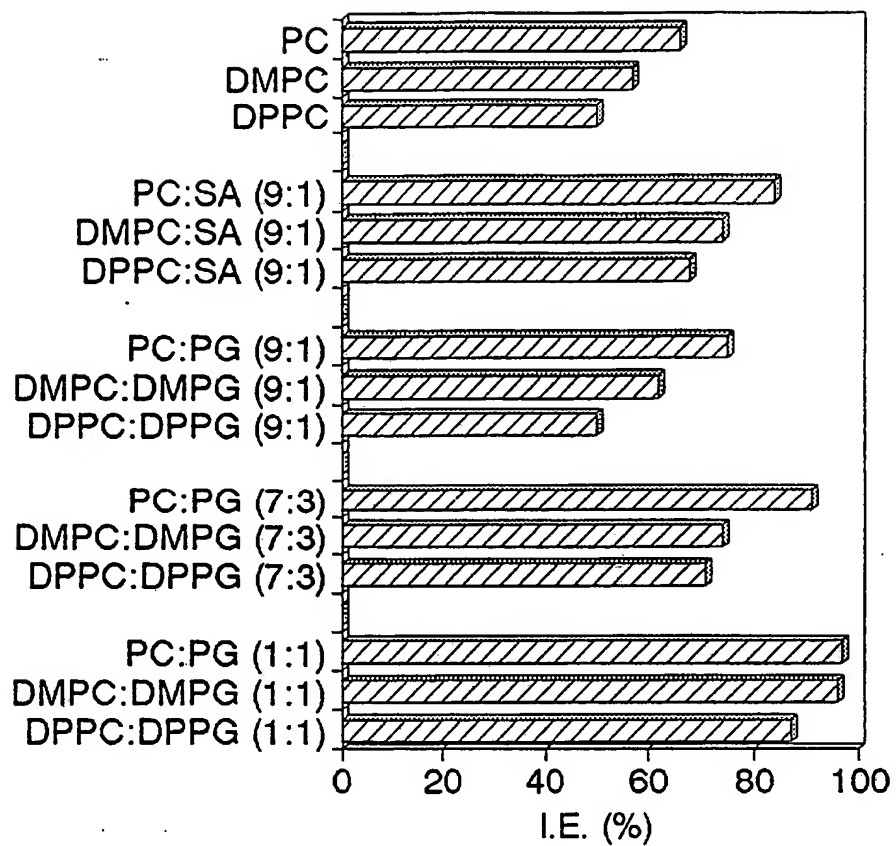


Fig. 1

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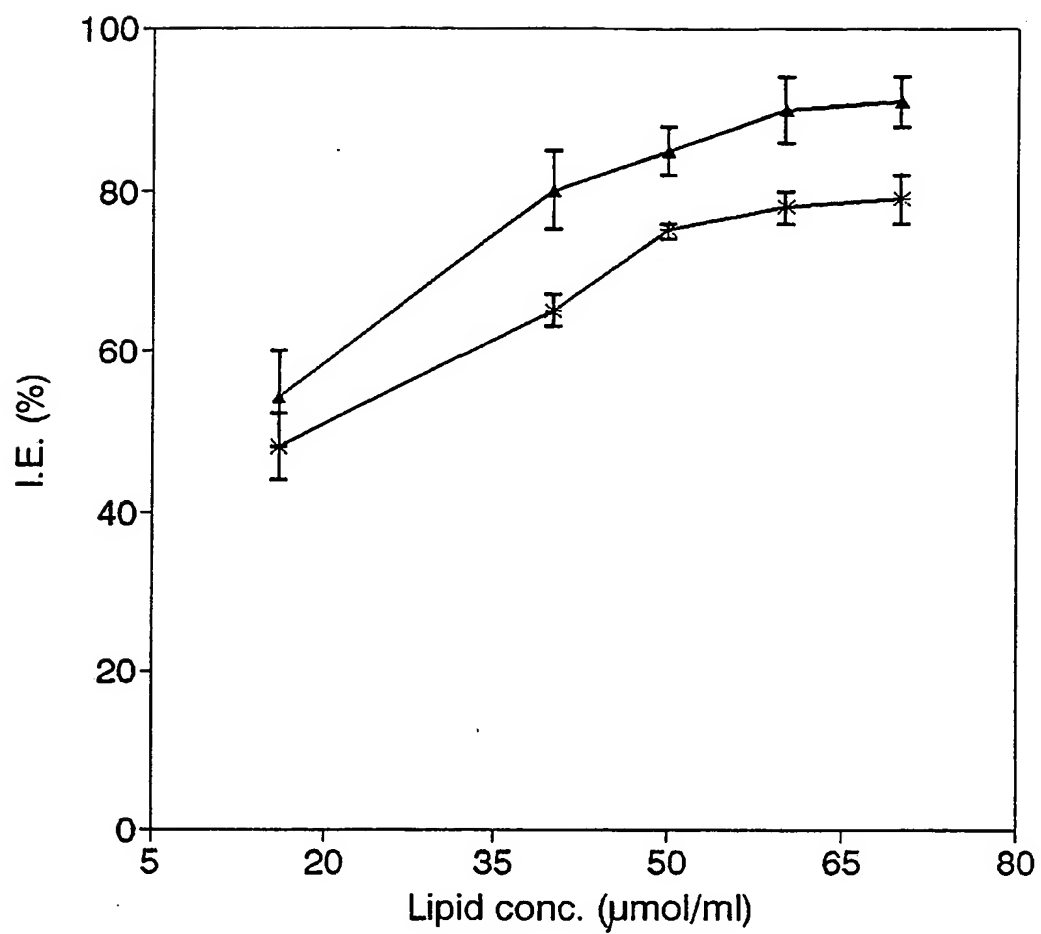


Fig. 2

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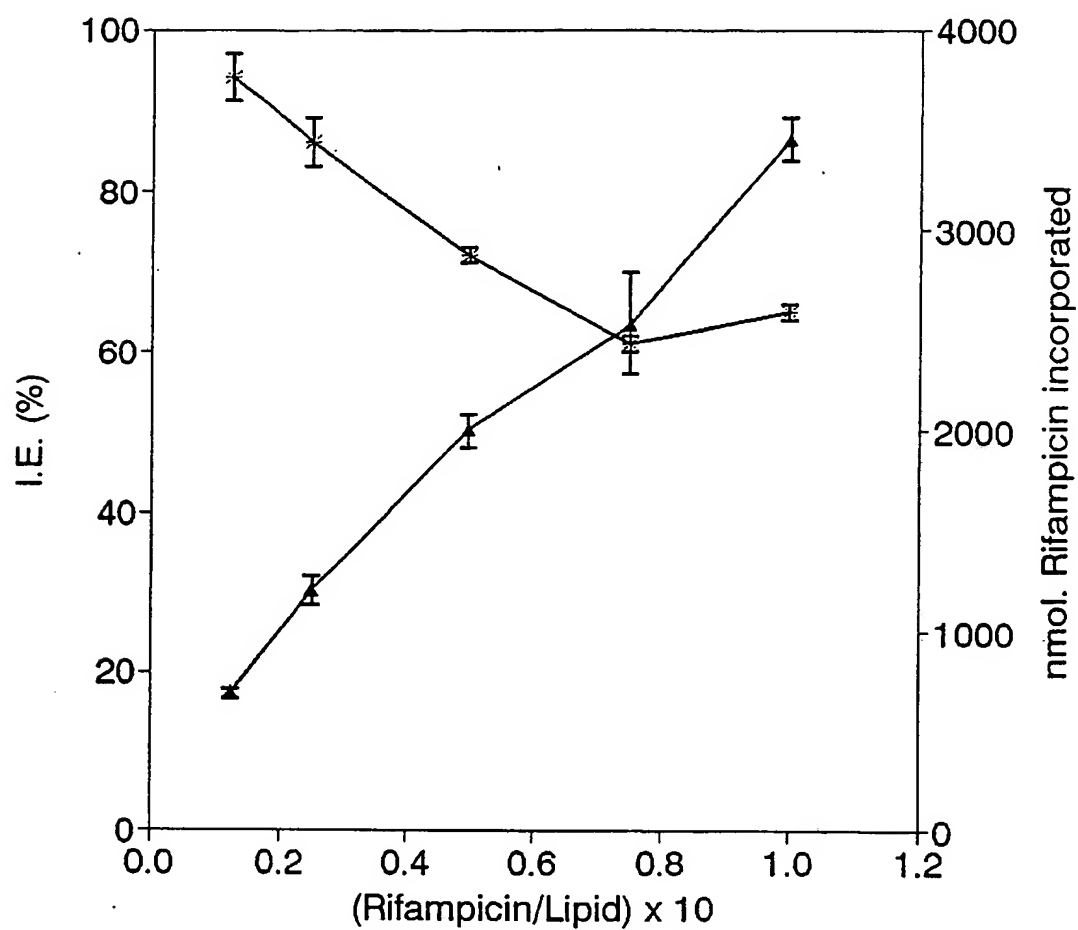


Fig. 3

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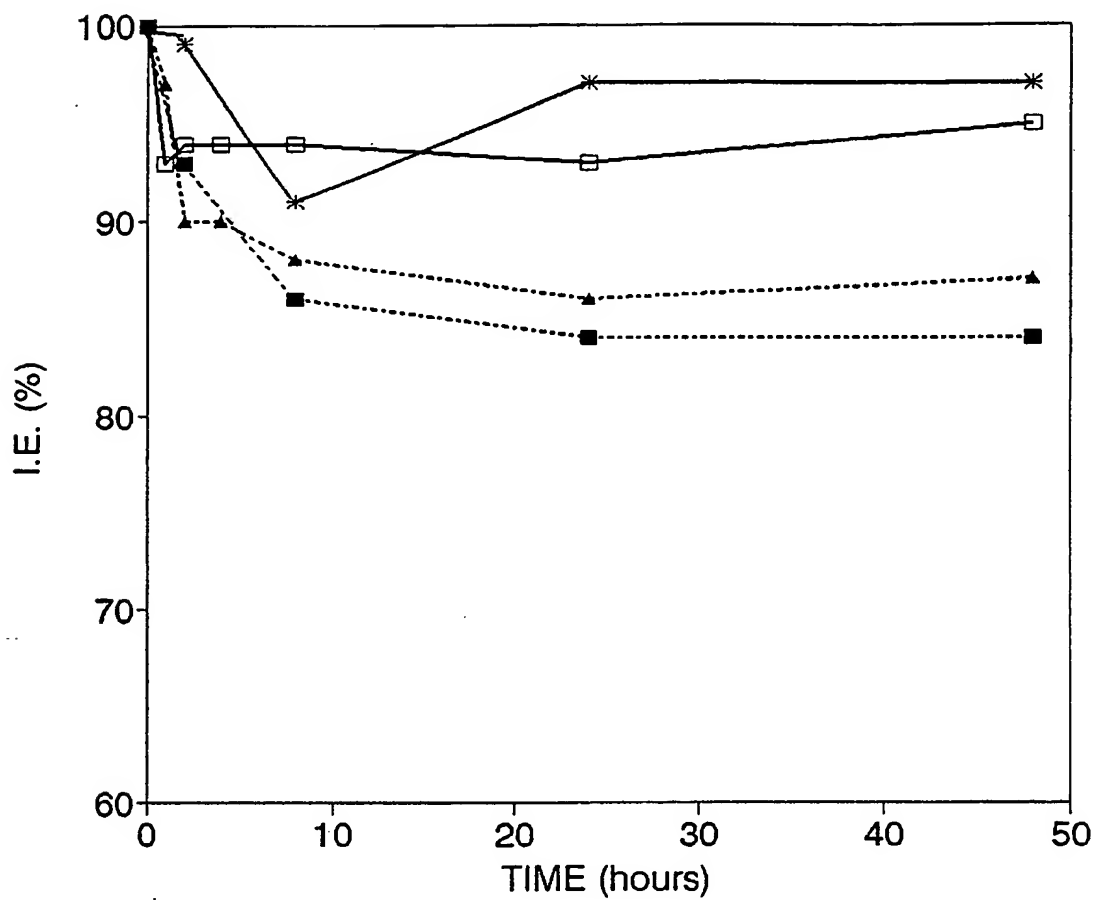


Fig. 4

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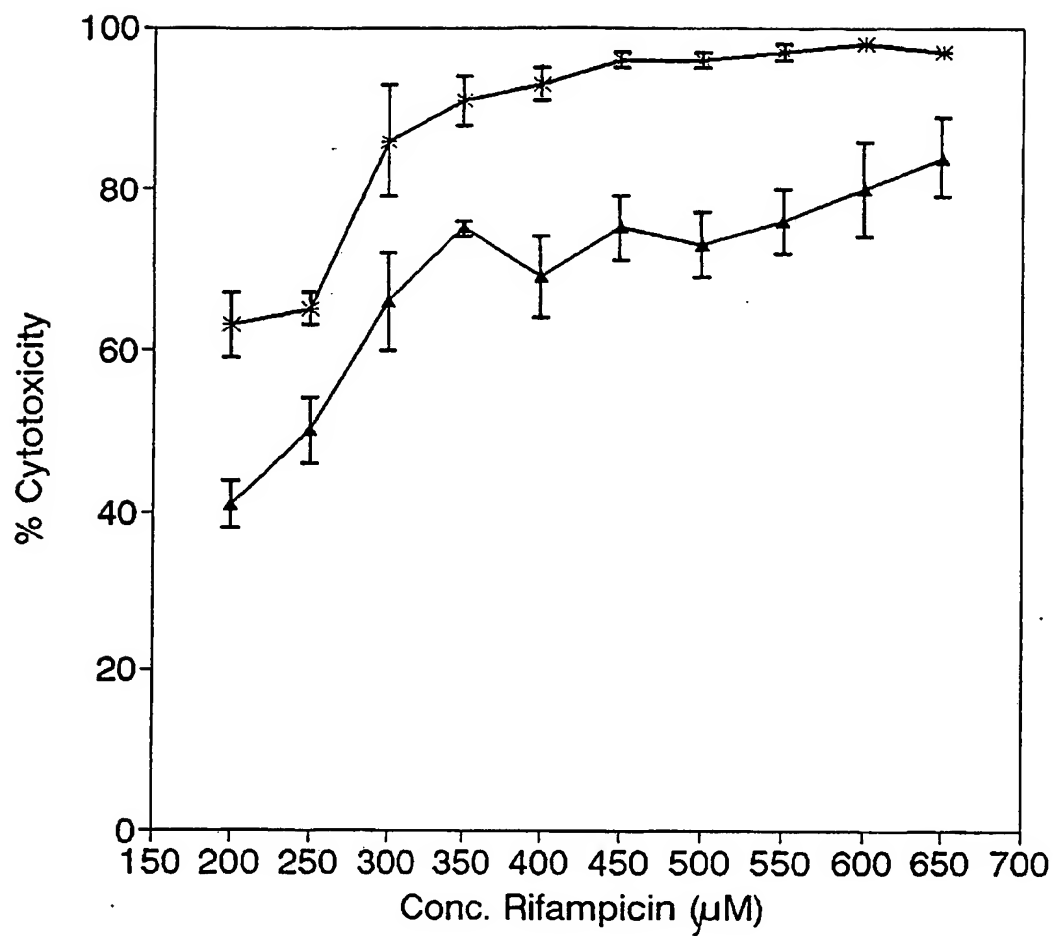


Fig. 5

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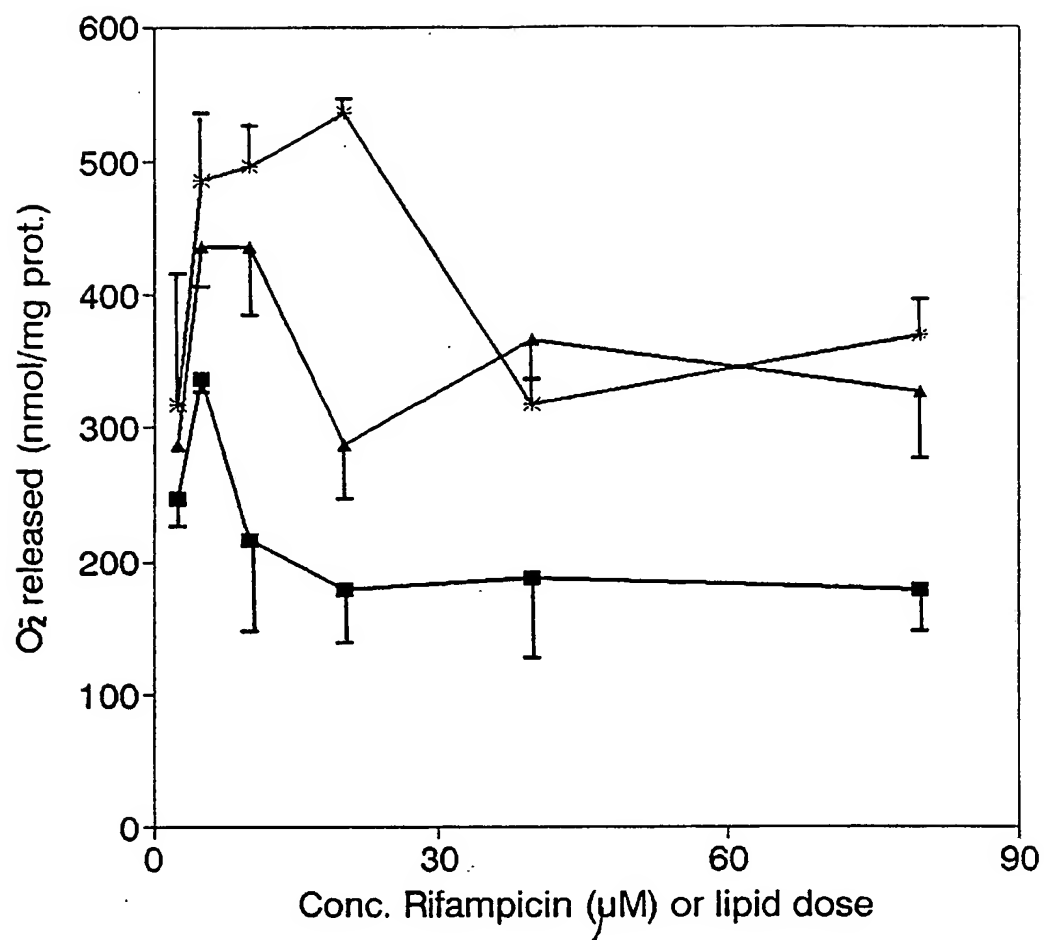


Fig. 6

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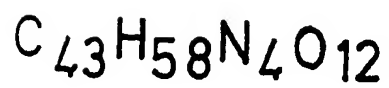
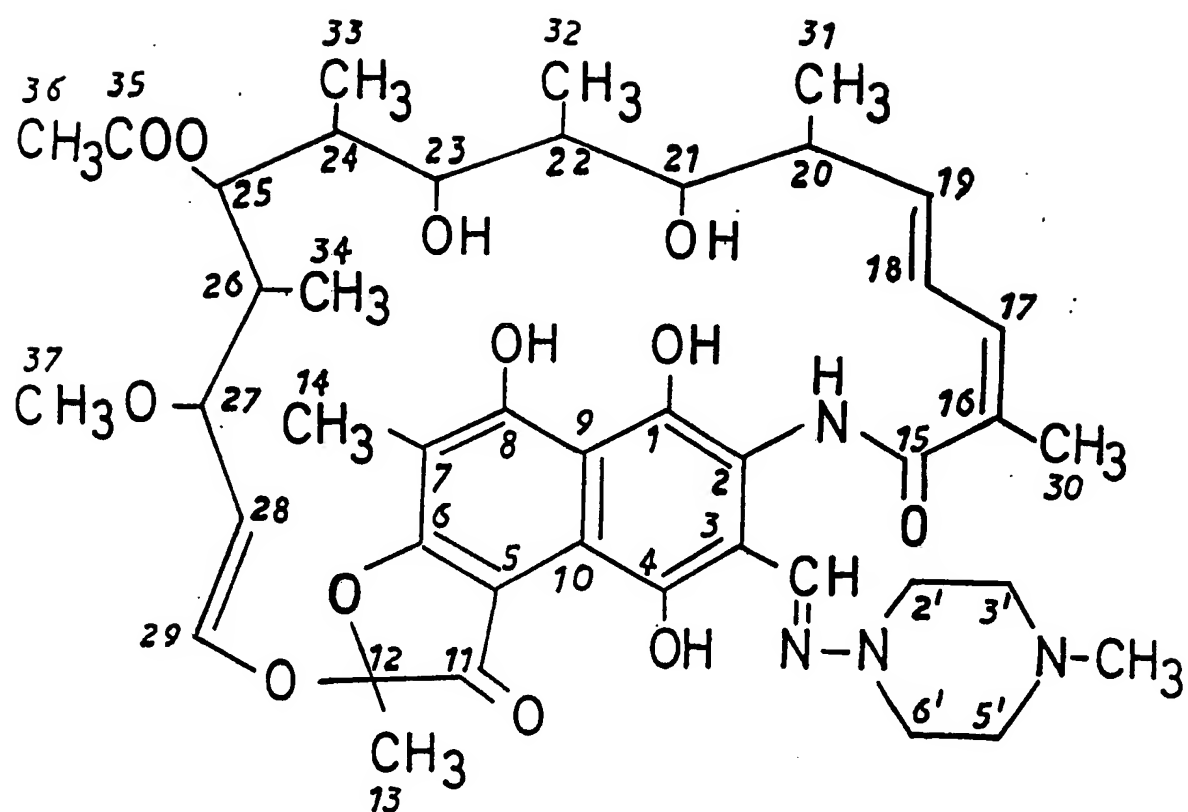


Fig. 7

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

PCT/PT 93/00002

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 A61K9/127; A61K31/395; A61K31/495		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	EP,A,0 178 624 (CIBA-GEIGY AG) 23 April 1986 see page 1, line 1 - line 6 SEE PAGE 9, LAST LINE see page 26 - page 27; example 5 ---	1-31
A	CHEMICAL ABSTRACTS, vol. 115, no. 26, 30 December 1991, Columbus, Ohio, US; abstract no. 287108t, A.A. AKHREM ET AL. 'effect of lipid composition of liposomes rifampicin incorporation into them' page 461 ;column 2 ; see abstract & VESTI AKAD. NAVUK BSSR, SER. KHIM. NAVUK vol. 1991, no. 5, pages 58 - 61 --- -/--	1-11,31
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
06 AUGUST 1993	17. 08. 93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	BENZ K.F.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category ^a	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 110, no. 22, 29 May 1989, Columbus, Ohio, US; abstract no. 199068w, H. SAITO ET AL. 'therapeutic efficacy of liposome-entrapped rifampin against mycobacterium avium complex infection induced in mice' page 408 ;column 2 ; see abstract	1-11,31
A	& ANTIMICROB. AGENTS CHEMOTHER. vol. 33, no. 4, 1989, pages 429 - 433 cited in the application	
A	--- CHEMICAL ABSTRACTS, vol. 99, no. 20, 14 November 1983, Columbus, Ohio, US; abstract no. 163956z; A. YA. TSYGANENKO ET AL. 'preparation and low-temperature storage of rifampicin-containing liposomes' page 340 ;column 1 ; see abstract	1-11,31
A	& ANTIBIOTIKI (MOSCOW) vol. 28, no. 8, 1983, pages 577 - 581 cited in the application -----	

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SA 73982

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EP-A-0178624	23-04-86	AU-B- 590221	02-11-89
		AU-A- 4871385	24-04-86
		CA-A- 1260393	26-09-89
		JP-A- 61097215	15-05-86
		US-A- 4971802	20-11-90
